

MYELOS.002DV2

PATENT

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: O'Brien, John S. et al.	)	Group Art Unit 1818
Appl. No.	: 08/484,594	)	
Filed	: June 7, 1995	)	
For	: USE OF PROSAPOSIN AND NEUROTROPHIC PEPTIDES DERIVED THEREFROM	)	
Examiner	: Robert Hayes, Ph.D.	)	

SECOND DECLARATION OF JOHN S. O'BRIEN, M.D.

Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

I, John S. O'Brien, M.D., declare and state:

1. I am an inventor of the above-identified application and am familiar with the specification, claims and file history thereof.
2. I have read the Office Action dated March 4, 1997 and participated by telephone in the interview conducted on May 7, 1997.
3. Enclosed herewith as Exhibit A are data showing the effect of a saposin C-derived peptide on TNF- $\alpha$ - and IFN- $\gamma$ -mediated apoptosis. CG4 cells were induced to differentiate into oligodendrocytes for 2 days in DMEM-N1 medium. The medium was then changed to DMEM (no fetal calf serum) and cells were treated with 200 ng/ml TNF (panel 1) or IFN- $\gamma$  (panel 2), either alone or in the presence of saposin C-derived peptide (TXA) or prosaposin. Dead cells

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were assayed by the trypan blue exclusion assay 24 hours later. As shown in Exhibit A, both peptide and prosaposin inhibited TNF- $\alpha$ - and IFN- $\gamma$ -mediated oligodendrocyte cell death.

4. Exhibit G of my previous declaration shows that thermal hypoalgesia in the rat diabetic neuropathy model was prevented by the TXA peptide. Subsequent morphometric analysis of myelinated axons in these rats has shown that diabetes induced a shift in the frequency histogram towards medium and small axons. This shift was attenuated by the peptide. Thus, the peptide inhibited the loss of large myelinated fiber neurons in diabetic neuropathy.

5. It is well known in neurobiology that neural function can be retained even if up to 90% of spinal cord motor neurons and up to 97% of dopaminergic neurons in the substantia nigra are lost.

6. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: 6/3/97

By: John S. O'Brien, M.D.

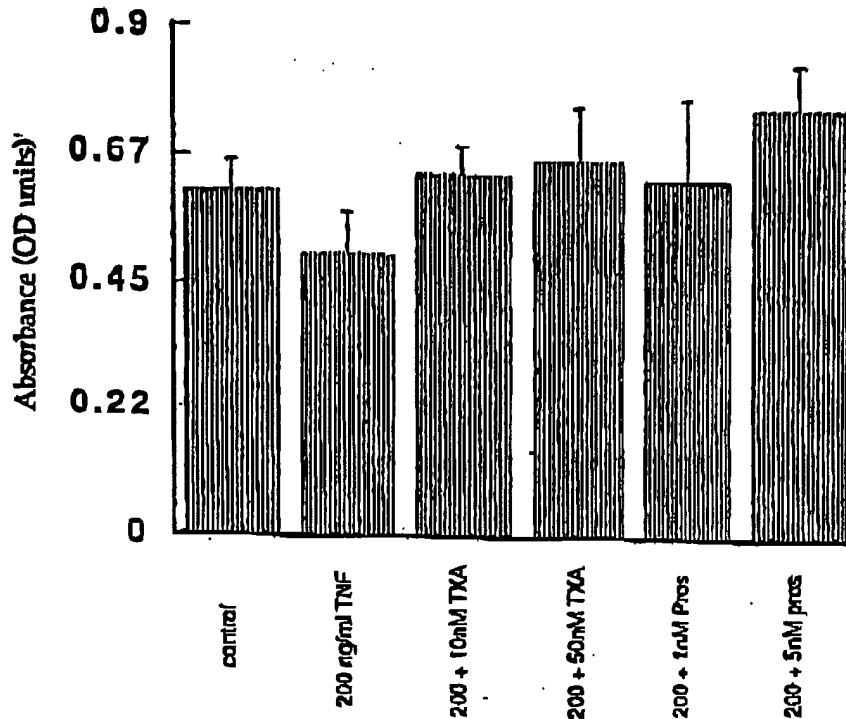
John S. O'Brien, M.D.

K58-0014  
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Appl. #08/484,954 Filed: 6/7/95  
By: J.S. O'Brien, et al. MYELOS.002DV2

## EXHIBIT A

*Undifferentiated Oligodendrocytes (G4 cells)*  
Effect of Prosaposin and TX14(A) on  
TNF-alpha-induced Death



Effect of Prosaposin and TX14(A) on  
IFN-gamma-induced Death

